Original paper

Expression pattern of ISL-1, TTF-1 and PAX5 in olfactory neuroblastoma

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Olfactory neuroblastoma (ONB) is a rare neoplasm of the sinonasal area with neuroendocrine differentiation. ISL-1, TTF-1 and PAX5 are transcription factors that are frequently upregulated in tumors showing neuroendocrine differentiation. The aim of our study was to evaluate these markers in a group of ONBs.

We included 11 ONBs from 4 large university hospitals. Immunohistochemical expression of TTF-1, PAX5 and ISL-1 was evaluated.

TTF-1, ISL-1 and PAX5 were expressed in 3/11 cases (27.27%, h-score: 3-45), 7/11 cases (63.64%, h-score: 23-200), and in 3/11 cases (27.77%, h-score 3-85), respectively. The patient with the strongest PAX5 reactivity exhibited an aggressive clinical course with rapid dissemination to the spine and death shortly after the diagnosis.

No significant correlation in the expression of PAX5 and TTF-1 ($\rho = 0.43$; $p = 0.18$) was observed.

ISL-1 is widely expressed in tumors with neuroendocrine differentiation and therefore of limited value in their differential diagnosis. TTF-1 positivity does not exclude the diagnosis of primary ONB, although usually only a small percentage of cells are positive.

PAX5 expression is infrequent (27.27%) in ONB; however, if present it can be associated with a very aggressive clinical course.

Key words: olfactory neuroblastoma, esthesioneuroblastoma, ISL-1, TTF-1, PAX5.

Introduction

Olfactory neuroblastoma (ONB), also known as esthesioneuroblastoma, was originally described in 1924 by the French physicians Berger and Luca in 1924. With its frequency of 0.4/million/year [1], it accounts for 3% of all sinonasal malignancies [2].

ONB is believed to be derived from olfactory epithelium, but its pathogenesis is largely unknown. Data on viral pathogenesis in some cases of animal ONB were not confirmed in humans [3]. ONB is frequently hormonally active and can thus result in paraneoplastic syndromes [4].

ONB presents with neuroendocrine differentiation, and primary as well as metastatic tumors can mimic other tumor entities with this differentiation pattern. Therefore, a continuous search for new markers that broaden differentiation with other neuroendocrine tumors is ongoing. Recent evidence shows that ISL-1, TTF-1 and PAX5 occur in a large percentage of neuroendocrine tumors [5, 6, 7, 8, 9]. However, the frequency of their expression and their expression pattern have not been studied in ONB.

No data are available about the functional relationship at the molecular level of TTF-1, PAX5 and ISL-1 in the literature. Also no relationship in their immunohistochemical expression has been studied despite their frequent presence in neuroendocrine tumors.

The aim of our study was to evaluate immunohistochemical expression of TTF-1, ISL-1 and PAX5 in a cohort of ONB collected from 4 large university hospitals in order to show their potential diagnostic usefulness. Additionally we assessed the potential reciprocal relationship between expression of these proteins in order to give some suggestions about the potential correlation in their biological activity and neoplastic transformation.

Material and methods

Patients

Databases of the departments and institutes of pathology in 4 large university hospitals were searched for the diagnosis of ONB or esthesioneuroblastoma. The retrieved cases were sent to the central laboratory (Medical University of Gdansk) for reevaluation. It was performed according to the criteria defined by the WHO classification of head and neck tumors.

Immunohistochemistry

Expression of PAX5, TTF-1 and ISL-1 was evaluated by immunohistochemistry. For TTF-1 and PAX5 ready-to-use antibodies from Dako (Denmark) were used and the staining was performed using an automated tissue stainer (Dako, Denmark). The analysis of ISL-1 (1 H9, Abcam, 1 : 1500) was performed manually.

Intensity of immunohistochemical reaction was evaluated using the h-score system. In h-score, the intensity of reaction and the percentage of positive cells were multiplied and summed up ($1\times$ n%$+2\times$n%$+3\times$ n% = y), i.e., the score ranged from 0 to 300. Each case was evaluated for each protein by two experienced pathologists (P.C. and A.G.).

Statistics

Spearman's rank correlation test was performed using Statistica 12 in order to evaluate the correlation in expression of ISL-1, PAX5 and TTF-1.

Results

Patients

The study group included 11 cases of ONB: 4 males and 7 females, aged 20-86 (mean: 52.3).

Basic clinicopathological characteristics of the patients are given in Table I.

Expression of TTF-1

TTF-1 was expressed in 3 cases (27.27%). Expression was nuclear and occurred in 1-27% of cells. In all cases there were at least single cells with strong immunoreactivity; the h-score of positive cases were in the range 3-45. Representative staining is shown in Fig. 1.

Expression of ISL-1

ISL-1 was expressed in 7/11 cases (63.63%). Expression was nuclear and it was observed in 15-90% of cells. The h-score of positive cases ranged between 23 and 200. Representative staining is shown in Fig. 2.

Expression of PAX5

PAX5 was expressed in 3/11 (27.27%) cases. One of them showed only single weakly positive cells (h-score: 3). The other tumors showed higher expression of PAX5 (16% and 35% of positive cells, h-score: 24 and 85, respectively). The patient with the highest level of PAX5 expression developed lymph node metastases at the primary diagnosis, and these recurred shortly afterwards with dissemination to the spine. She died 3 months after the initial diagnosis. PAX5 immunohistochemical staining is shown in Fig. 3.

Correlations in the expression of the markers

We did not observe statistically significant correlation between the expression of investigated proteins. However, taking into account the low number of cases it can be stated that there is a trend toward a moderate correlation between expression of TTF-1 and

Table I. Basic clinicopathological data of ONB cohort

Sex	Male	4 (36%)
	Female	7 (64%)
Age (years) 20-86	Average	52.3
	Median	50.5
Grade	I	2 (18%)
	II	5 (45%)
	III	3 (27%)
	IV	1 (9%)

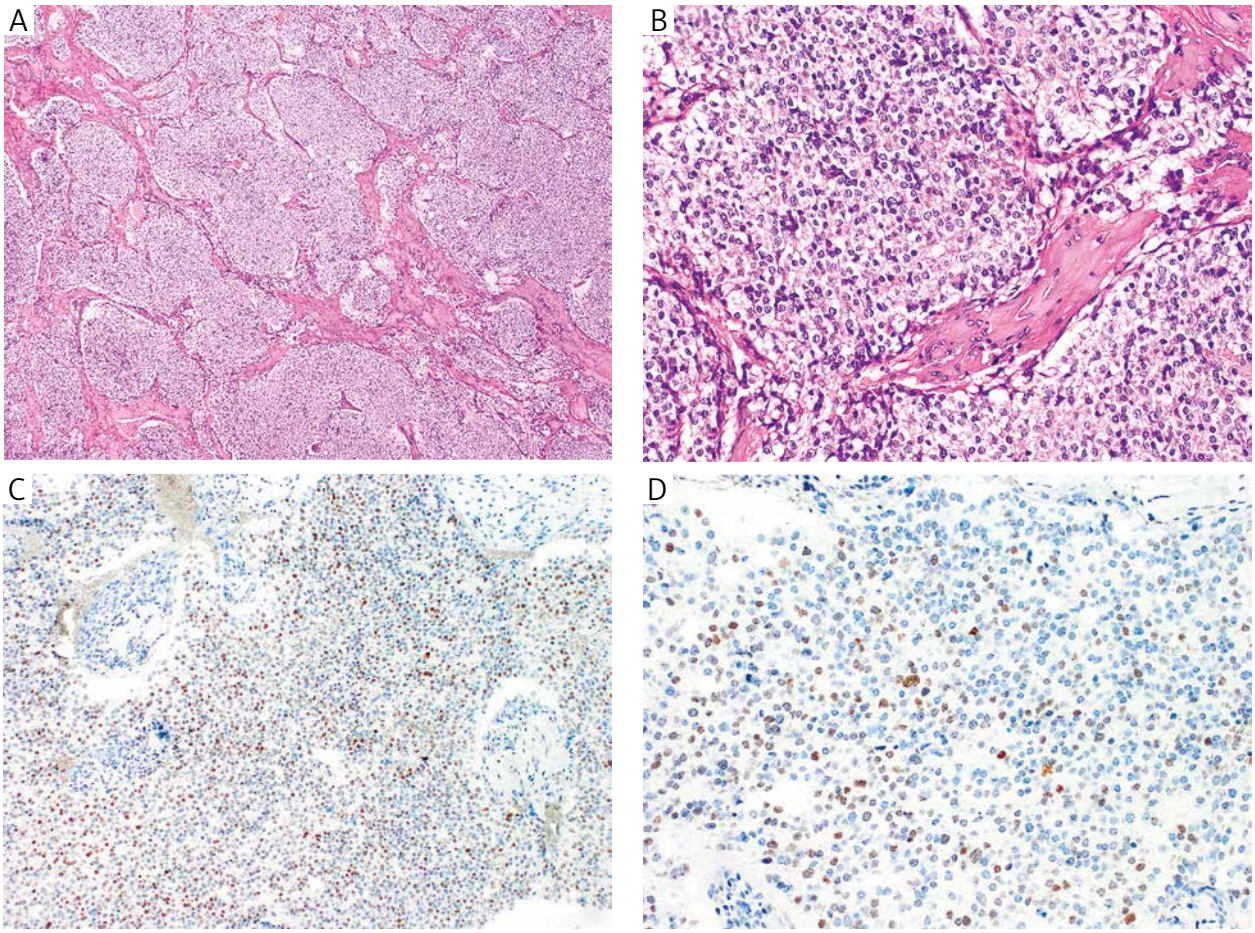

Fig. 1. Nests of olfactory neuroblastoma (A, HE, magnification 40×) with small, vesicular nuclei (B, HE, magnification 200×) showing moderate to strong immunohistochemical reaction to TTF-1 (C, magnification 100×; D, magnification 200×)

PAX5. The Spearman rank correlation test resulted in the following values for the investigated proteins:
- ISL-1 and TTF-1 ($\rho = 0.0029$; $p = 0.99$),
- ISL-1 and PAX5 ($\rho = 0.0828$; $p = 0.81$),
- TTF-1 and PAX5 ($\rho = 0.43$; $p = 0.18$).

Discussion

PAX5 is a member of the paired box (PAX) family of genes that are proteins involved in the regulation of development and differentiation of multiple tissue types [10]. PAX5 is a transcription factor that controls B-cell development and is present on B-cells from early to late stages of B-cell differentiation [11]. Initially it was believed to be a specific marker of B-cell neoplasms, but it has also been identified in many other types of tumors with neuronal and neuroendocrine differentiation [12, 13, 14].

PAX5 is activated in the mesencephalon during murine embryogenesis [10], and knock-out of this gene results in malformations of the midbrain [15]. The role of PAX5 in the development and function of the olfactory epithelium is not known.

We showed for the first time that PAX5 is expressed in a subpopulation of ONB (3/11; 27.27%). In one of them only 3% of cells showed a weak reaction, which from a diagnostic point of view is meaningless. Two other cases revealed stronger immunoexpression in 16 and 35% of cancer cells, respectively. This observation adds ONB to the large group of neuroendocrine tumors that may show PAX5 reactivity.

The patient with the highest PAX5 expression exhibited a very aggressive clinical course of disease: lymph node metastases at the diagnosis, rapid progression with spinal involvement and death 3 months after the onset of therapy. Unfortunately, the clinical course of the remaining two PAX5-positive cases could not be retrieved from the clinical files. Therefore, it is not possible to evaluate the role of PAX5 expression regarding the clinical course and outcome. Its evaluation in an independent and larger cohort of PAX5-positive ONBs may answer this question.

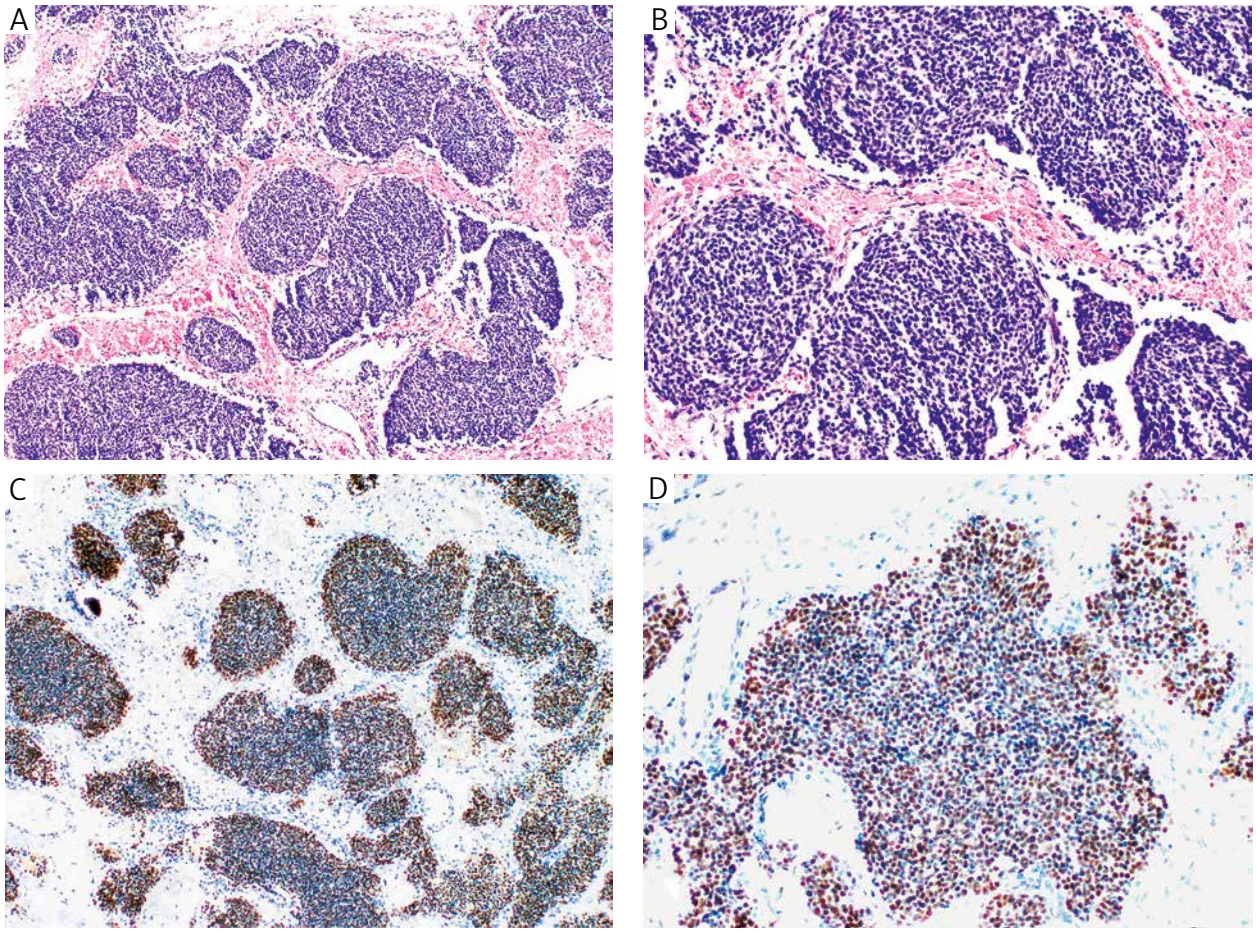

Fig. 2. Nests of monomorphic neoplastic cells (A, HE, magnification 100×; B, HE, magnification 200×) with moderate to strong expression of ISL-1 (C, magnification 100×; D, magnification 200×)

TTF-1 is a marker of pulmonary and thyroid origin of tumors [16]. However, up to 40% of high-grade neuroendocrine tumors of extrapulmonary origin show expression of this transcription factor [8, 9]. In the human fetal brain, TTF-1 expression has been reported in the infundibular anlage and ependymal and subependymal cells of the third ventricle [17]. Its role in the development and function of the olfactory epithelium remains unknown.

In the primary tumors, 3/11 cases were positive, although in one case only single cells were strongly positive (h-score = 3), while the other two cases were positive in 20-27% of cells.

Some authors believe that TTF-1 expression excludes the diagnosis of ONB. However, we think that TTF-1 expression is just a marker of neuroendocrine differentiation, as it may be present in a substantial group of neuroendocrine tumors at various locations. Therefore, it does not exclude *per se* diagnosis of ONB. On the other hand, in none of our cases was strong, diffuse expression observed, so the differential diagnosis with the most common neuroendocrine malignancy, small cell carcinoma of the lung, which is usually strongly TTF-1 positive, should not be problematic in this setting.

ISL-1 was originally described by Karlsson *et al.* as a protein that binds to an insulin gene enhancer and regulates its expression [18]. Its crucial role in the development and maturation of the endocrine cells in the pancreas was confirmed [19]. Initially expression of ISL-1 was believed to be restricted to neuroendocrine tumors of pancreatic origin. However, recent data show that it can be expressed in the majority of neuroendocrine tumors of extrapancreatic origin, including Merkel cell carcinomas, pulmonary small cell neuroendocrine carcinoma, medullary thyroid carcinomas, paragangliomas/pheochromocytomas, and adrenal neuroblastomas [6].

Recently we have described the expression of ISL-1 in 44% (11/25) of medulloblastomas (Czapiewski, Pathology Research and Practice, in press). In the developing brain ISL-1 promotes striatonigral and represses striatopallidal genetic programs [20]. The role of ISL-1 in the development and function of olfactory epithelium is not known.

In a report regarding ISL-1 expression in a small group of 4 cases of ONB, only 1 case was positive [6]. In our cohort of ONB patients expression of ISL-1 was present in the majority of cases (63.63%). All of the positive cases were at least moderately immuno-

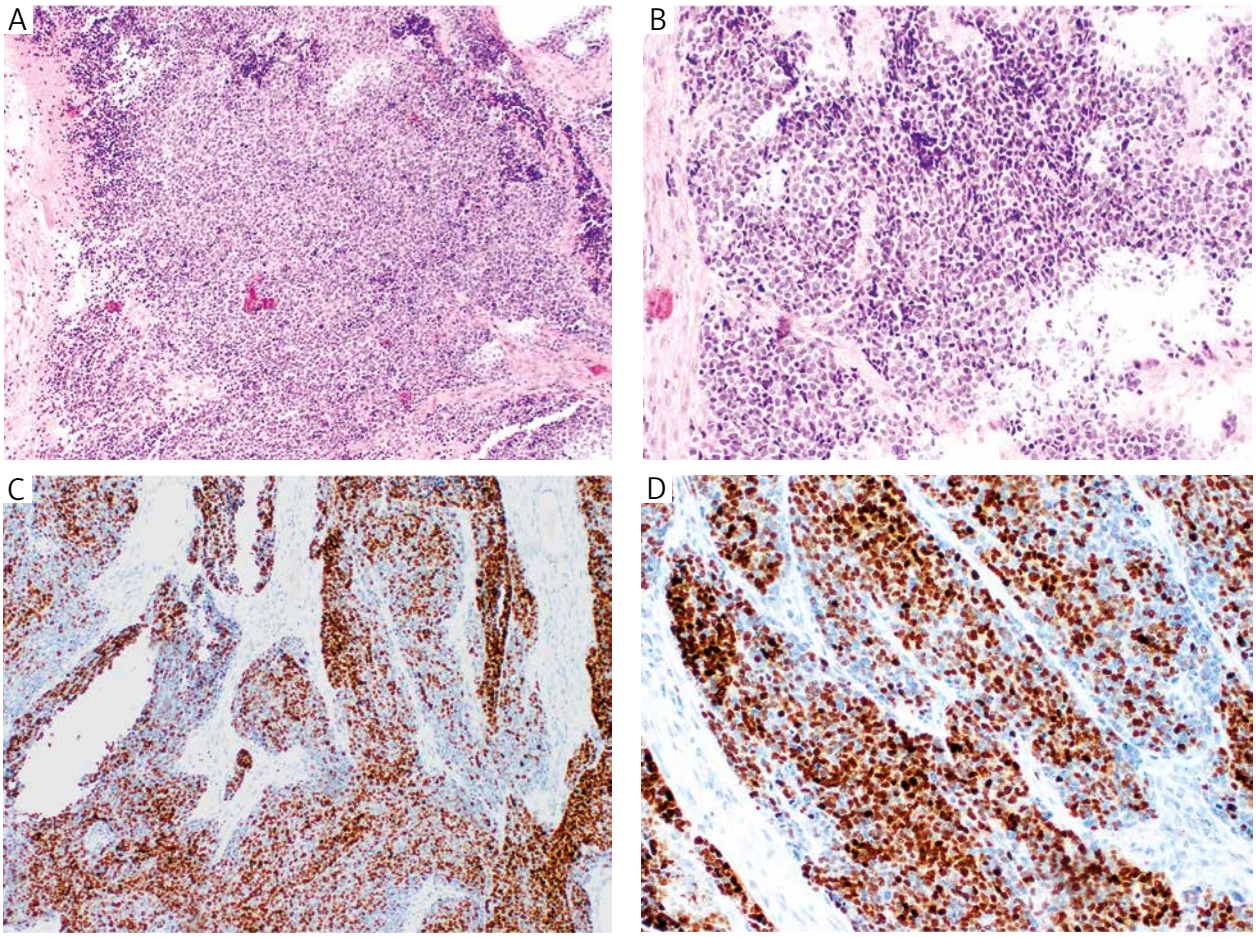

Fig. 3. Cohesive nests of olfactory neuroblastoma cells (A, HE, magnification 100×; B, HE, magnification 200×) with strong expression of PAX5 (C, magnification 100×; D, magnification 200×)

reactive, and in 3 cases immunoexpression was very strong (h-score 170-200).

These results increase the group of tumors with neuroendocrine differentiation and at the same time make this marker even less specific for the diagnosis of pancreatic neuroendocrine tumors than previously thought. Positive reaction with this antibody does not rule out and does not confirm the diagnosis of ONB. This can be of diagnostic value in metastases of neuroendocrine neoplasm to the sinonasal area. We do not know the expression of this marker in ONB metastases, but probably it may also be misleading under these clinical circumstances.

To sum up, ISL-1 can show a positive reaction in ONB and should be taken into consideration in the differential diagnosis of neuroendocrine tumors of the sinonasal area.

We did not observe a statistically significant correlation in expression of PAX5, ISL-1 and TTF-1 in our cohort of ONB. However, we detected a trend toward a moderate correlation between expression of PAX5 and TTF-1. The lack of significant correlation may be due small number of cases. It cannot be excluded that no relationship exists. On the other hand, this may be a real connection which could point to a common role of TTF-1 and PAX5 in biological activity and neoplastic transformation of these two proteins. However, no data confirming this dependency in neuroendocrine or any other tumor exist. This aspect requires further studies on larger ONB cohorts and in other neuroendocrine tumors.

Conclusions

Our results shed light on the diagnostic role of ISL-1, PAX5 and TTF-1 in ONB. Frequent ISL-1 positivity in ONB increases the spectrum of ISL-1 positive tumors with neuronal and neuroendocrine differentiation and proves that ISL-1 should not be considered as a marker of pancreatic origin in pancreatic tumors.

TTF-1 positivity does not exclude the diagnosis of ONB. PAX5 expression is infrequent in ONB, but strong reactivity can be associated with high grade tumors and very aggressive clinical course with rapid skeletal dissemination.

PAX5 and TTF-1 show a trend toward a correlation in expression which might be secondary to a yet

undescribed molecular cooperation during the process of neoplastic transformation of cells.

The authors declare no conflict of interest.

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